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Kniest Dysplasia: Dr. W. Kniest, His Patient, the Molecular Defect

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Kniest dysplasia is a severe chondrodysplasia caused by the defective formation of type II collagen. We report about Dr. Kniest, who first described the condition in 1952, and his patient, who, at the age of 50 years is severely handicapped with short stature, restricted joint mobility, and blindness but is mentally alert and leads an active life. Molecular analysis of the patient's DNA showed a single base (G) deletion involving the GT dinucleotide at the start of intron 18 destroying a splice site of the COL2A1 gene. This is in accordance with molecular findings in other patients with Kniest dysplasia and confirms, in the original patient, that the disorder is caused by small inframe deletions often due to exon skipping as a result of COL2A1 splice site mutations. Am. J. Med. Genet. 69:79–84, 1997.

KEY WORDS: chondrodysplasia; type II collagenopathy; COL2A1 mutation; splice site defect; phenotype-genotype correlation

INTRODUCTION

In 1952, Dr. Wilhelm Kniest[1], then chief resident of the Children's Hospital of the University of Jena, in Thuringia. Keeping a lifelong interest in bone dysplasias, notably the disease which now bears his name [Kniest and Leiber 1977, Kniest 1979], he retired from his position as head of the Naumburg Children's Hospital in 1985 and presently lives an active life in Naumberg. He is still working with the local transfusion service, keeps up with modern developments in pediatrics, is interested in local and world politics and spends his leisure time hiking and visiting the art centers of the world.

On the occasion of his 75th birthday in 1994 an entire page of the local newspaper was devoted to him as a respected physician and beloved citizen quoting "In the entire adult population of Naumburg there is hardly a person who has not been cared for by Dr. Kniest."

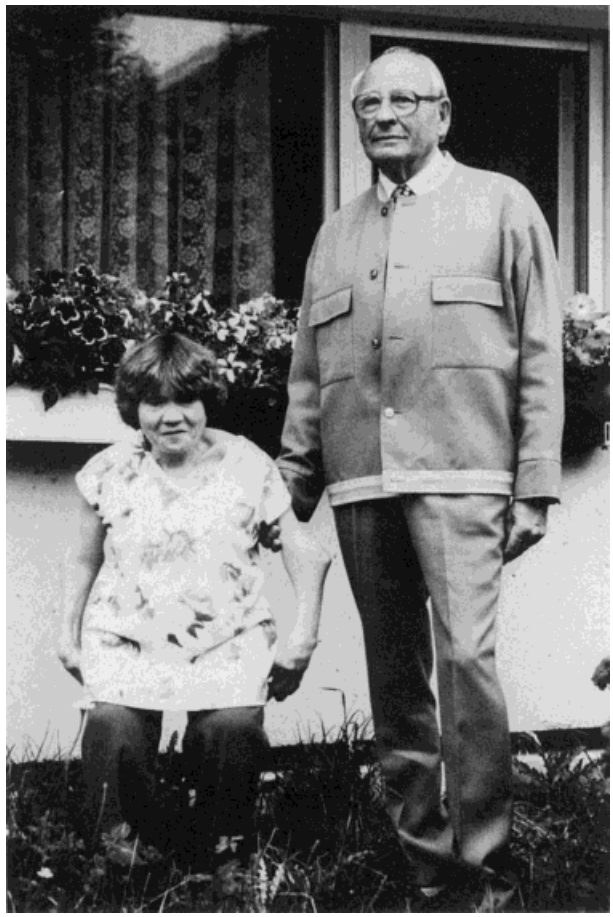

Fig. 1. Wilhelm Kniest and his patient in 1995.

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[1] Wilhelm Kniest was born on October 25, 1919 in Obersuhl/Hassia as the son of a merchant. He went to school in Eisenach, a small city in Thuringia, and studied medicine at the universities of Berlin, Leipzig, Wien and Würzburg. In 1941, the war interrupted his medical career. In 1946 he was able to resume his education with Kleinschmidt, one of the most prominent German pediatricians of his time, who introduced him to pediatrics and recommended him to Jussuf Ibrahim, head of the Children's Hospital of Jena/Thuringia, where he was trained to become a pediatrician.

On January 1, 1953, Kniest was appointed chief physician of the newly founded children's hospital in Naumburg, a small city

Thuringia, published the case history of a 3½-year-old girl with "skeletal changes showing a certain relationship to classical chondrodystrophy but differing in many of its manifestations" [Kniest, 1952]. Publication followed an intensive search of the literature and correspondence with authorities such as Otto Ullrich, Hans-Rudolf Wiedemann in Bonn, and Erich Häßler in Chemnitz, which convinced the author that the field of "enchondral dysostoses," quoting Ullrich, was "an awful mess" that badly needed clarification. His publication contributed to the delineation of a disorder that was formerly confused with other chondrodystrophies and is now known to be one of the type II collagenopathies.

It is testimony to the warmth and humanity of Wilhelm Kniest that the relationship with his patient persists to the present day (Fig. 1) enabling us to meet her, describe the further development of her disease and the underlying molecular defect.

CLINICAL REPORT

R.W. was born in 1946 as the second of three children of healthy, nonconsanguineous parents. Her length at

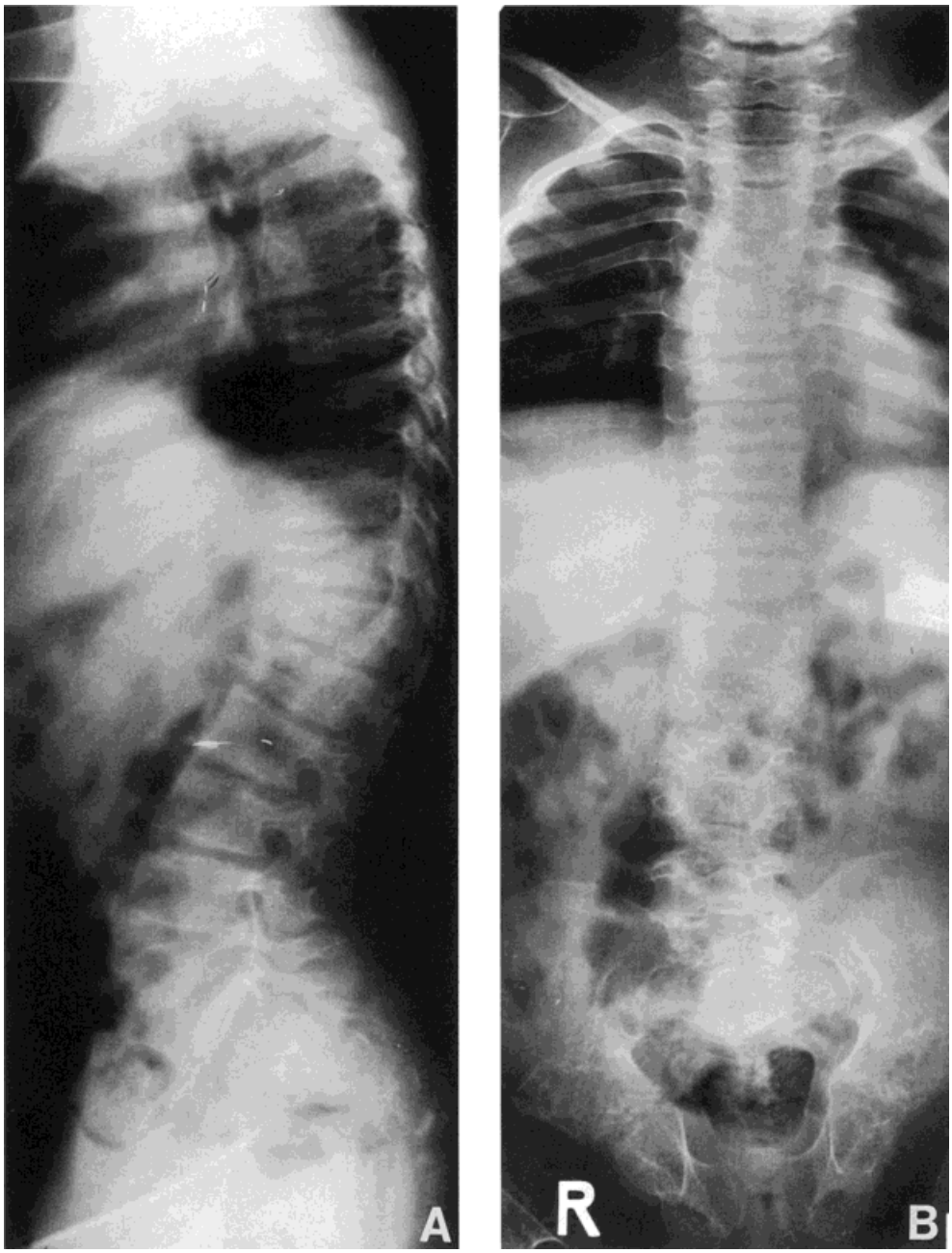

Fig. 2. **A,B:** Four and a half years. The vertebral bodies are flattened with anterior wedging in the lower thoracic and upper lumbar spine.

birth was not recorded but she was noted to have short limbs and a relatively large head with prominent eyes. Her mental development was normal but she first sat without support at 2 years after being immobilized in a plaster cast for 4 month because of "dislocated hips." When seen by Kniest at the age of 3.5 years, she was able to stand and walk a few steps with support. Her height was 77 cm (−5 S.D.), her face flat with protruding eyes, and her thorax broad and short. The large joints were prominent with reduced mobility.

During the next years subcondylar tibial osteotomies were performed to correct the valgus deformities of her knees. Plaster casts were applied to treat the kyphoscoliosis. Severe myopia was noted. At the age of 11 years retinal detachment and glaucoma developed resulting in blindness. A photograph in a follow-up publication by Kniest shows her at the age of 28 years with a height of 115 cm, multiple joint contractures, and kyphoscoliosis [Kniest, 1979].

Mental development remained normal. Living in a home for the handicapped she learned Braille and worked at home fitting parts in electric instruments. She married at the age of 42 years and lives presently with her husband in their own home. Her present appearance is depicted in Figure 1.

SKELETAL DEVELOPMENT

Radiographs are available from ages 4½ and 29 years showing platyspondyly with anterior wedging of the vertebral bodies, broad ilia with hypoplasia of the basilar portions, broad and short femoral necks, shortened tubular bones with flared ends, and irregular articular surfaces (Figs. 2–4).

MOLECULAR DEFECT

COL2A1 gene analysis was done as formerly described [Winterpacht et al., 1993, 1994] using a set of 46

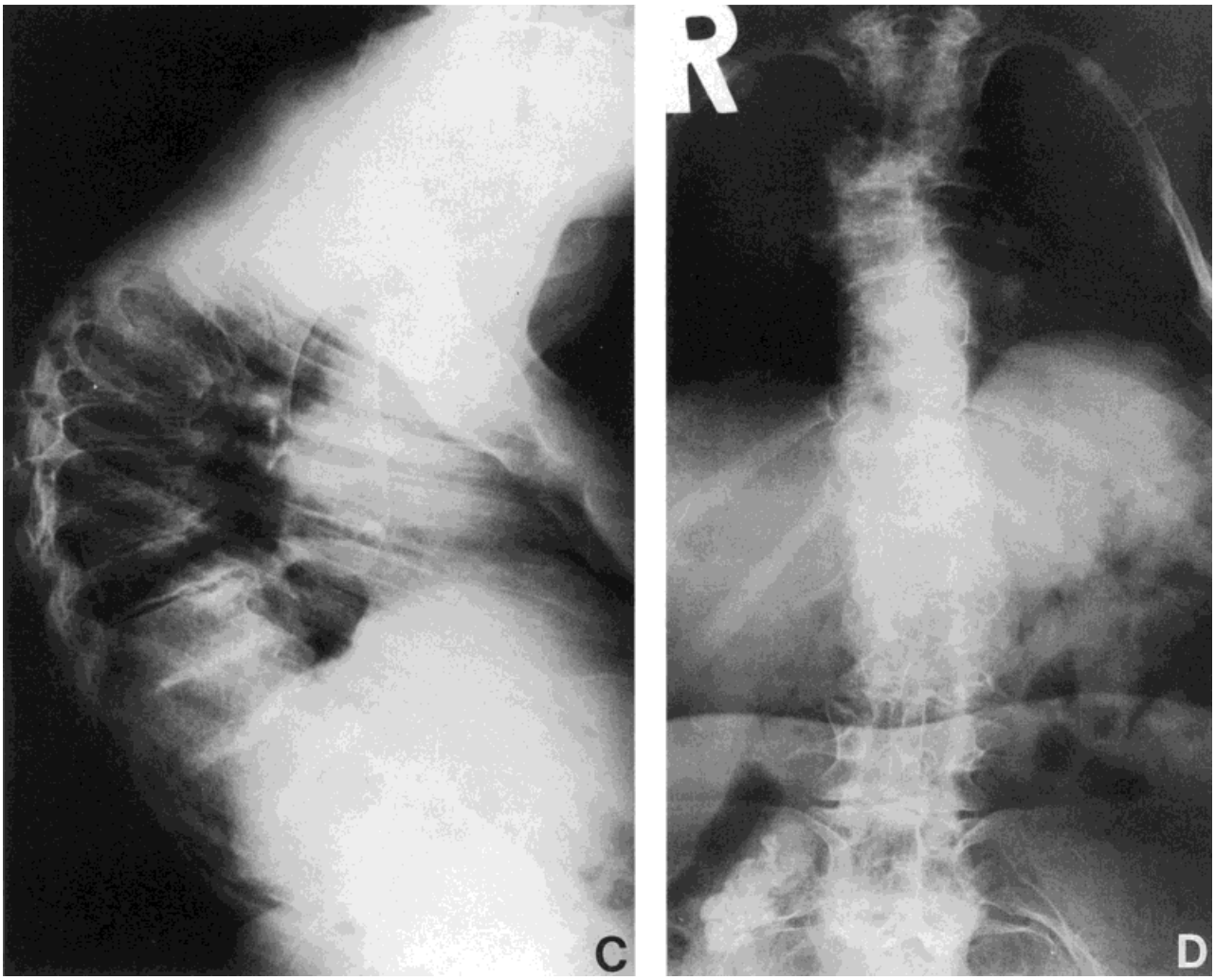

Fig. 2. **C,D:** Twenty-nine years. The thoracic kyphosis has increased but there is only mild progression of the scoliosis.

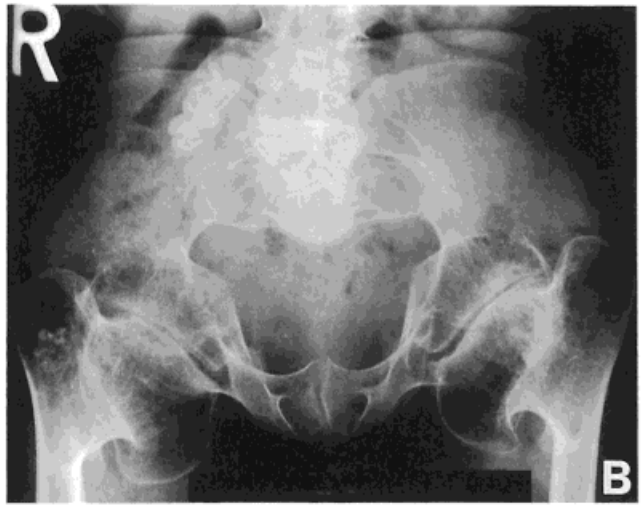

Fig. 3. **A:** Four and a half years. The ilia are short in their craniocaudal dimension. The capital femoral epiphyses show only minimal specks of ossification. The femoral necks are short and broad with a medial protrusion. Femora are short with wide distal portions, slightly irregular metaphyseal margins and irregularly ossified distal epiphyses. The proximal tibial growth plates have a shallow V-shaped configuration. **B:** Twenty-nine years. The ilia are short with shallow acetabula. The femoral heads are large and in varus position with irregular articular surfaces.

primer pairs designed according to the exon/intron boundaries published by Ala-Kokko and Prockop [1990]. These primers allow amplification of all 54 exons including their flanking splice sites. Single strand conformation polymorphism (SSCP) analysis [Orita et al., 1989] was used to screen the PCR products for the presence of mutations leading to an additional band for exon 18 on the SSCP gel. After sequencing we identified a single base (G) deletion involving the highly conserved GT dinucleotide at the start of intron 18 and thus destroying the 5′-donor splice site (Fig. 5, left). This most likely results in skipping of the complete exon 18 from the mature mRNA producing a type II procollagen chain shortened by 18 amino acids (Fig. 5, right).

DISCUSSION

Kniest's original patient shows the severity and slow progression of the disease with short stature involving both trunk and extremities, marked joint contractures, thick joints, and moderately severe kyphoscoliosis. The ocular changes were severe with myopia, retinal detachment, glaucoma, and final blindness. The palate was intact and hearing was normal. Mixed hearing loss and cleft palate have been described in other patients [Siggers et al., 1974; Kim et al., 1975; Pennock et al., 1979; Bogaert et al., 1994; Spranger et al., 1994] and may be the cause of delayed speech development and mental retardation [Siggers et al., 1974; Castroviejo, 1977]. Kniest's patient is of normal intelligence and leads a fulfilled and meaningful professional and family life. The same can probably be said for the 43-year-old patient published by Kim et al. [1975], the mother of a 12-year-old girl.

Molecular analysis demonstrated a single base deletion destroying a splice site of the COL2A1 gene. Other mutations found in Kniest dysplasia include a 28 bp deletion causing a 5′-splice site mutation and resulting in skipping of exon 12 [Winterpacht et al., 1993], a 3′-splice site point mutation resulting in the deletion of six amino acids from exon 21 [Winterpacht et al., 1994] and from exon 34 (Winterpacht et al., in preparation) two identical out of register deletions (disrupting the Gly-Xaa-Yaa pattern) in exon 12 observed in two unrelated patients [Bogaert et al., 1994], a 18 bp in frame deletion in exon 49 [Winterpacht et al., 1996], and the present case describing the original patient with a single base deletion affecting a 5′-splice site most likely resulting in skipping of exon 18. A patient with a Gly (103)→Asp exchange classified as Kniest dysplasia [Wilkin et al., 1994] turned out to develop a SEDC phenotype (personal communication).

Fig. 4. **A:** Four and a half years. The tubular bones are short with wide proximal and distal ends and slightly irregular epiphyseal ossification centers. **B:** Twenty-nine years. The fingers are fixed in flexion showing wide ends with irregular articular surfaces and narrow joint spaces.

Taken together, the data suggest that Kniest dysplasia is caused by small inframe deletions in the COL2A1 gene (with possible hot spot around exon 12), often due to exon skipping as a result of splice site mutations. An essential factor to get the severe and rather specific phenotype seems to be the incorporation of the shortened α-chain into the fibrils (due to the intact C-terminal region). The negative effect on fibril formation may be explained by the resulting misalignment of cross-linking sites and problems during helix formation.

Although we were not able to analyze the patient's cartilage and thus provide definitive proof of the proposed exon skipping and incorporation of the shortened proα_1(II) chains into fibrils, we think, that the molecular defect in Kniest's original observation confirms the genotype-phenotype correlation established for other cases with Kniest dysplasia. Long-term follow-up of the patient demonstrates the severe clinical consequences of the mutation but also gives testimony to the courage and determination of an individual afflicted by this crippling condition.

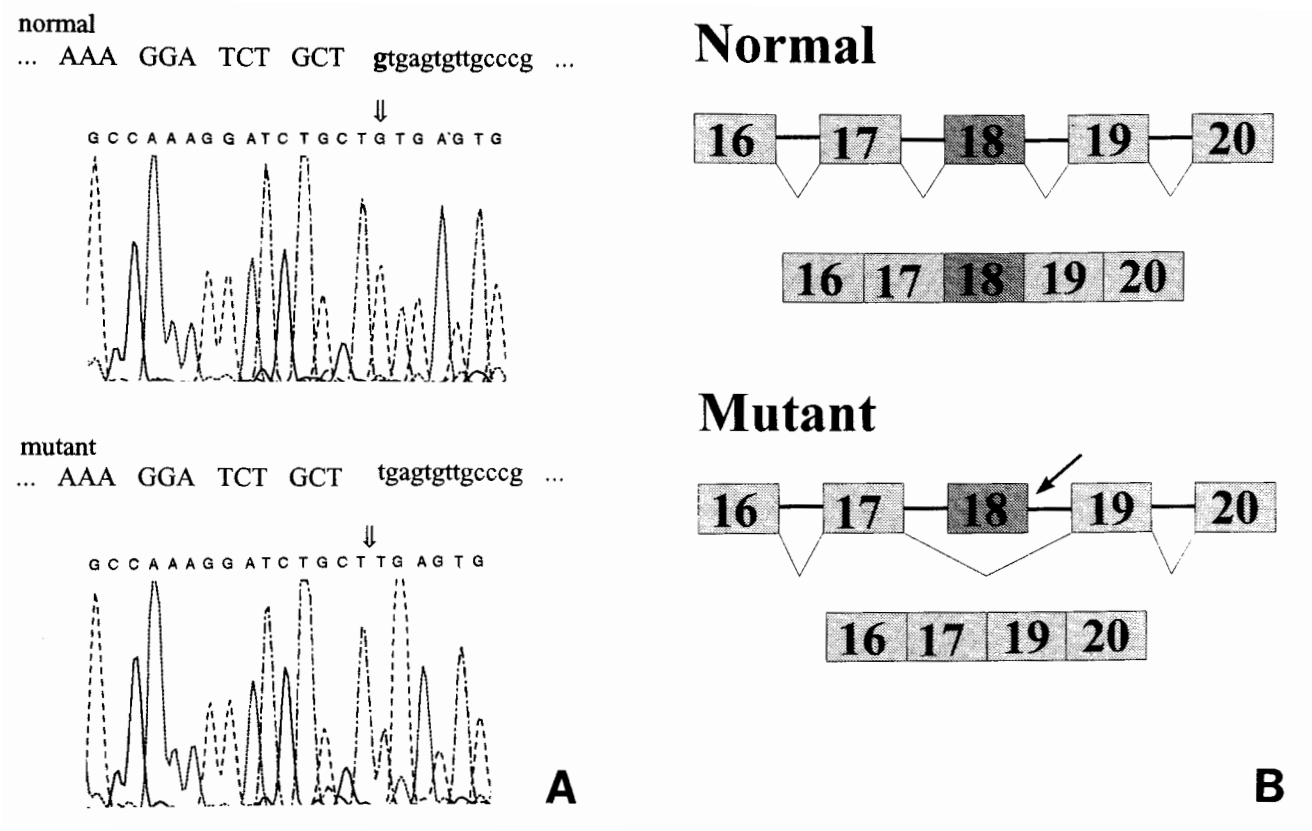

Fig. 5. **A:** Single base (G) deletion involving the highly conserved GT dinucleotide at the start of intron 18 and thus destroying the 5′-donor splice site. **B:** Proposed skipping of the complete exon 18 from the mature mRNA as result of the mutation.

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